

# AN ALTERNATIVE VIEW OF CERTAIN TAXONOMIC CRITERIA USED IN THE ENTOMOPHTHORALES (ZYGOMYCETES)

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Long-standing unresolved controversies about the various generic classifications of the entomopathogenic Entomophthorales require a review of the validity and the relative weights of the morphological and other taxonomic criteria used to construct a useful classification for this group. Major morphological criteria used in the Batko and Remaudière classifications are evaluated here in a survey of the taxonomic distributions of these characters and the significance of the irregularities in their distributions.

The number and nature of nuclei in the primary spores, the branching of sporophores, and the mode of discharge of primary spores are regarded as the major characters suitable for delimiting genera. The presence of rhizoids and (to a limited extent) the shapes of primary spores are significant secondary characters. The presence of cystidia, mode of resting spore formation, and presence of capillary secondary sporophores are not regarded as characters of generic importance. The conidial or sporangial nature of primary spores is discussed, as are difficulties in the interpretation and applications of the term *zygospore* and *azygospore*.

The applications of the generic names used in the Batko and Remaudière classifications are reviewed in light of the findings reported here. The validity of both *Strongwellsea* and *Entomophaga* is upheld, and the sense of *Erynia* is restricted to those species having branched sporophores and uninucleate bitunicate primary spores which do not form secondary spores on capillary sporophores. A nomenclatural problem is noted which indicates the synonymy of *Zoophthora* and *Erynia* if these genera are not retained as separate as proposed in the Remaudière classification. *Tabanomyces* is regarded as a synonym of the nematophagous genus *Meristacrum*.

## INTRODUCTION

A far-reaching revision of the entomopathogenic genera of the Entomophthorales (Zygomycetes) proposed by Batko (1964a-e, 1966; Batko and Weiser 1965) has spawned considerable controversy among the students of these fungi. Even though there is now wide agreement that it is unsatisfactory to continue classifying approximately 150 diverse species in the single genus *Entomophthora* and to establish segregate genera or of the circumscriptions of these genera.

King and Humber (1981) offer a basic reconsideration of the major morphological characters of this group and discuss their taxonomic significance. While King and Humber evaluate the basic strengths and weaknesses of the Batko classification, they consider that no definitive reworking of the genera would be appropriate without a final resolution of the long-standing problem of delimiting *Conidiobolus* Brefeld from *Entomophthora* sensu lato.

More recently, however, a major reworking of Batko's generic classification by Remaudière and Keller (1980) — hereafter referred to as the Remaudière classification — resulted in generic assignments for some species which led to no manifest increase in the biological homogeneity of the recognized genera. Many taxonomic decisions in the Remaudière classification, and particularly those regarding the validity and importance of various characters used to construct classifications of these fungi, were presented without explanation and, in turn, have triggered further controversy and alternate views on classification (Ben-Ze'ev and Kenneth 1981a; Humber 1981a).

The time is appropriate to examine thoroughly those morphological and developmental characters which have been employed in the modern classifications proposed by Batko and by Remaudière and his colleagues. The values of these criteria are examined in the contexts of the available classification systems.

Indeed, a sense of the relative values of their chosen criteria does emerge from a consideration of the major schemes of the entomophthoralean genera and of the internal inconsistencies generated by the selection and ranking of the criteria accepted as valid in each of these generic schemes.

Despite its undisputed desirability, no general agreement regarding the generic classification of the Entomophthorales will be possible until the morphological and other criteria used in the past or being proposed now have been critically reviewed,

evaluated, and agreed upon. This paper is the first in a series discussing the merits and weaknesses of the Batko, Remaudière, and other emergent classifications (e.g., Ben-Ze'ev and Kenneth 1981a) in an attempt to find rational bases on which to build a generic scheme which (1) avoids the inconsistencies of the previous classifications of the Entomophthorales, (2) can accurately predict the natures of characters or aspects of the host-pathogen relationship, and (3) reflects what appear to be the evolutionary relationships among these fungi.

## TAXONOMIC CRITERIA: ANALYSIS AND CONCLUSIONS

### PRIMARY SPORES

#### Primary spores: Conidia or Sporangia?

Throughout this paper I refer to *primary spores* or *spores* rather than to the more conventions *conidium* or the less common *sporangium* or *sporangiole*. *Conidium* and *sporangium* are terms referring to specific developmental patterns; their use should be for purposes of accuracy and must be backed by sufficient demonstration of the mode of sporogenesis. The reference to any asexual propagules in the Zygomycetes as *conidia* has been questioned by the students of the Hyphomycetes and Coelomycetes, although there is no reason to believe *a priori* that conidia cannot be formed by these or any other lower fungi. If appropriate studies of the origins and fates of the various wall layers of spores (and sporophores) during sporogenesis and germination prove that, indeed, they are conidia (or sporangia), then these propagules should be referred to as such. Similar studies are also needed for the panoply of secondary spore types produced in the Entomophthorales.

Spores containing single nuclei and borne on branched (or simple, in *Strongwellsea* Batko & Weiser emend. Humber) sporophores have caused much interpretational difficulty. These spores have a layer which, in liquid, lifts away from the spore surface and balloons outward. This has been regarded variously as a gelatinous coat (e.g., *Entomophthora gloeospora* Vuillemin 1886) or, more frequently, as evidence for the sporangial nature of these fungi by regarding the detached layer as a sporangial wall enclosing a single sporangiospore (Thaxter 1888; Strong et al. 1960; Batko 1964b, 1974). This type of primary spore seems to be entirely restricted to the species of *Zoophtora* Batko, *Erynia* Nowakowski, and *Strongwellsea*. Kenneth (1877) claims that the outer wall of *Entomophthora turbinata* Kenneth [= *Neozygites turbinata* (Ken.) Rem. & Keller, a generic assignment which remains debatable] lifts away but admits (personal communication) that this feature is doubtful and required further investigation. If the spores of *E. turbinata* truly were bitunicate, (with a separable

outer wall layer), it would be the only instance of this character in a species with multinucleate spores.

Humber (1975; King and Humber 1981; Roberts and Humber 1981) notes ultrastructural evidence that the spore of *Strongwellsea magna* Humber, which has a separable outer wall layer, cannot be a monosporic sporangiole. The spore wall has no indigenous (sporangiospore) layer that is not shared with the sporophore itself. The separable layer is not a distinct (sporangial) wall, but represents only a separation of the outermost layer of the sporophore wall, all layers of which are continuous over the entire spore surface except for the papilla — which is covered only by an extension of the inner wall layer of the spore and sporophore (Humber 1975). This spore might be a true conidium, but this cannot be confirmed without also determining the fates of the wall layers during germination (see Dykstra 1974).

The only evidence for the occurrence of sporangia in the Entomophthorales comes from several species of *Basidiobolus* Eidam, in which there are numerous instances when the cytoplasm inside a cell usually but not always a spore) may be divided into several independent uninucleate units; *B. microspor* Benjamin (1962) provides the most striking examples of this behavior.

I use the terms *unitunicate* and *bitunicate* introduced by Remaudière and Hennebert (1980) and Remaudière and Keller (1980) to refer to spores on which a separable outer wall layer is either absent or present, respectively. These terms are used almost exclusively within mycology to refer to the morphology (and function) of asci in the Ascomycotina, and constitute a primary character dividing the (bitunicate) Loculoascomycetes from all other (unitunicate) classes of this subdivision (Ainsworth et al. 1973). The use of these terms, however, should be generally accepted for the Entomophthorales since they (1) are convenient, (2) respect their etymological derivations, (3) should not be in any way confused with their applications for the ascomycetes, and (4) in the case of bitunicate spores, would help suppress the notion that such spores are sporangial in nature.

## Morphology

The variety of shapes of primary spores in the Entomophthorales has been the source of much attention, particularly among those who have included nearly all entomopathogens of this group in the single genus *Entomophthora*. A series of artificial species groupings based on spore morphology have been proposed primarily to aid species identification (Lakon 1919; Hutchison 1963; Gustafsson 1965; MacLeod and Müller-Kögler 1970, 1973; MacLeod *et al.* 1976; Waterhouse 1975; Zimmermann 1978). None of these authors, however, intended their groupings to have taxonomic value. Remaudière and Keller (1980), however, chose the morphology of primary spores to be the main criterion for their generic classification.

Several genera do have characters of spore morphology which are unique to themselves. One of the strongest and most valid criticisms of the Batko classification, in fact, was its separation of the species having campanulate spores (with relatively flat bases and a strongly apiculate apex) into *Entomophthora* *sensu stricto* and *Culicicola* Nieuwland depending upon the absence or presence of rhizoids. Remaudière and Keller (1980) rejected the significance of rhizoids for defining the genera, and redefined *Entomophthora* to include only species with this distinctive campanulate spore shape and associated mode of spore discharge (see MacLeod *et al.* 1976; Samson *et al.* 1979).

The spores of *Triplosporium* (Thax.) Batko (*nom. gen. conserv. prop.*, see Humber *et al.* 1981; = *Neozygites* Witlaczil) are variable in shape, but most often are round to slightly elongate and have a truncate rather than a conical or rounded papilla. As circumscribed by Batko (1964c), the spores of *Culicicola* species could be either campanulate (like those of *E. muscae* (Cohn) Fres.) or obpyriform with a smooth apex and rounded papilla; species with the former type were restored to *Entomophthora* *s.str.* while those with the latter type of spores were transferred to *Conidiobolus* by Remaudière and Keller (1980). King (1976b, 1977) regards *Conidiobolus* species to have round to pyriform primary spores, and notes the similarity of several entomopathogenic *Entomophthora* (*sensu lato*) species with *Conidiobolus*. Remaudière and Keller (1980) extended King's concept of *Conidiobolus* by transferring all entomopathogenic species with rounded to pyriform spores to that genus. The species of *Entomophaga* Batko have pyriform spores with a rounded apex and closely resemble the spores of *Conidiobolus* species. These two genera are unambiguously differentiated, however, by both the morphology and stain reactions of their nuclei (Humber 1981b).

The primary spores of *Zoophthora* Batko *sensu* Remaudière and Hennebert (1980) (= *Zoophthora* subg. *Zoophthora* Batko *sensu* Ben-Ze'ev and

Kenneth 1981a) are rather cylindrical and usually taper apically to a blunt cone or a poorly defined point; just above the conical papilla, the spore flares to a very slight (but not always apparent) shoulder. The spores of *Erynia* Nowakowski species (see Remaudière and Hennebert 1980) display the greatest shape variation of any genus in the Remaudière classification. They range from elongate fusiform with a marked curvature and a shallow rounded papilla to somewhat cylindrical to ovoid or fusiform spores with rounded apices and papillae canted away from the spore axis. The spores of *Strongwellsea* species fit well within the range of morphological variation found in *Erynia*, a fact which seems to have motivated Remaudière and Keller (1980) to merge this genus into *Erynia*. The spores of *Empusa caroliniana* Thaxter, which Remaudière and Hennebert (1980) transferred to *Erynia*, are obovoid to pyriform, a shape corresponding more with those of *Erynia* spores than with any other genus.

## Karyology: Number and Type of Nuclei

Early studies noting the taxonomic importance of entomophthoralean nuclei have been largely ignored (Vuillemin 1895; Cavara 1899a-b), so that no surpassing importance was attached to the number of nuclei in primary spores until the proposition of Batko's classification (1964a-d, 1966; Batko and Weiser 1965), which necessarily drew upon the observations of earlier workers regarding this cytological detail.

One of the most significant characters used by Batko (1964b) to establish *Zoophthora* was that of uninucleate primary spores, a character which is readily detectable and seems to be correlated well with the other characters of this genus. *Strongwellsea* Batko & Weiser (1965) was also described as having uninucleate spores but was clearly distinguished from *Zoophthora* by its simple sporophores and unique habit. *Erynia* Nowak. was split from *Zoophthora* by Remaudière and Hennebert (1980) and is circumscribed so as to allow multinucleate primary spores even though *E. caroliniana* is the only such species included in *Erynia* by Remaudière and his colleagues. As discussed at length below, the morphological characters of this species do not appear to belong in this genus, and its probable misclassification is clearly indicated by its status as the only species with multinucleate spores in this large grouping of species with uninucleate spores.

As is the case with branched sporophores (see below), there is a very low but natural incidence of abnormally developed primary spores. The spores of *Zoophthora*, *Erynia*, and *Strongwellsea* receive very nearly the entire cytoplasmic contents of the terminal cell of the sporophore. In species with branched sporophores there are, occasionally, larger than usual volumes of cytoplasm containing two (or three) nuclei which are isolated in a branch by its basal septum. The spore subsequently formed from this branch will

be both markedly larger than normal and contain the extra nucleus or nuclei. Such exceptionally rare spores are aberrant and cannot be used to invalidate the significance of uninucleate spores. Similarly, they do not justify the inclusion in *Erynia* (all of whose other species have uninucleate primary spores) of *E. caroliniana* (whose spores are always multinucleate and whose other major morphological characters do not match with the other species of *Erynia*).

Three genera are noted by Batko (1964a-b, 1974) to have plurinucleate spores. The spores of several *Entomophthora* species (sensu Remaudière and Keller 1980) contain 4-6 nuclei; those of *E. culicis* (Braun) Fres. are characteristically binucleate (or occasionally trinucleate) while those of *E. muscae* (Cohn) Fres. contain 5-8 nuclei. *Triplosporium* (= *Neozygites*) was described by Batko (1964b) to have quadrinucleate spores. Of the eight species included in *Neozygites* by Remaudière and Keller (1980), seven have 4-nucleate spores; *Entomophthora turbinata* Kenneth (1977) has spores with 5-7 or more nuclei. This deviation from the quadrinucleate condition of all other species in this genus indicates that close scrutiny must be given to the generic assignment of this relatively little studied fungus. On first examination, the only marked similarities of *E. turbinata* with the other species of *Triplosporium* are its simple sporophores and ovoid resting spores with a jet black epispore. Batko (1974) incorrectly indicated that the primary spores of *Massospora* Peck are binucleate; in fact, they vary from 1-6 (or more nuclei), but most commonly contain 2-3 nuclei (Soper 1974, 1981).

The number of nuclei in spores of *Culicicola* species (as originally described by Batko 1964c-d) varied from pluri- to multinucleate, but really represent a bimodal distribution with either a few nuclei or many in the primary spores. This bimodal variation perfectly parallels the dispersal of *Culicicola* species for other reasons to either *Entomophthora* s.str. (plurinucleate) or *Conidiobolus* (multinucleate).

The taxonomic importance of nuclear morphology has not been appreciated until now even though some authors such as Batko (1964a-b, 1966; Batko and Weiser 1965) have drawn attention to the staining of nuclei in lactophenol cotton or aniline blue or other stains, or to their general size and appearance, but never realized their potential taxonomic value. The large nuclei of *Basidiobolus* species, for example, have been the subjects of numerous cytological studies.

Humber (1981b) finds that nuclei of *Conidiobolus* species (sensu King 1976b) have relatively small nuclei with a prominent central nucleolus but no obvious heterochromatin; these nuclei remain unstained or only very weakly differentiated in lactophenol/aniline blue, aceto-orcein, or a number of other nuclear stains. The species of *Entomophthora* and most of the entomopathogenic genera have large

nuclei with prominent heterochromatin but no large central nucleolus; these nuclei may be differentiated in lactophenol/aniline blue and are strongly and rapidly differentiated in aceto-orcein and other nuclear stains. This karyological criterion has proven to be of inestimable value for diagnostic work, particularly for distinguishing species of *Conidiobolus* from *Entomophaga* (whose primary spores are nearly identical in shape) or other genera with readily differentiated nuclei, and in determining incidences of double infections in insects when one of the fungi is a species of *Conidiobolus*.

#### Taxonomic Significance of Primary Spores

Batko's recognition of the generic significance of the number of nuclei (one, a few, or many) in primary spores has been widely embraced. The Remaudière classification, however, necessarily disregarded this character in order to place the greatest generic value on the shapes of primary spores. As will be made more apparent below, this dependence upon spore shapes is misplaced and leads to what seem to be several misclassifications which offer no improvement over earlier artificial schemes based on spore shapes and proposed without taxonomic or phylogenetic significance.

Nuclear cytology affords the only consistent and readily observed criteria which adequately distinguish the species of *Conidiobolus* from *Entomophthora* and its segregates (Humber 1981b). These criteria also uphold the validity of *Entomophaga* Batko for *E. grylli* (Fres.) Batko and several other species. *Entomophthora obscura* Hall & Dunn and *E. thaxteriana* (Petch) Hall & Bell were assigned to *Entomophaga* (Batko 1964d) but are now regarded as synonyms (see below); nuclear cytology confirms the transfer of *Entomophthora obscura* to *Conidiobolus* by Remaudière and Keller (1980).

The unitunicate or bitunicate nature of primary spores appears to be perfectly correlated with the nuclear number. With the sole unconfirmed exception of *E. turbinata*, all known bitunicate spores also contain a single large nucleus. Except for two species of *Massospora* in which uninucleate primary spores can occur (Soper 1974), all unitunicate spores are pluri- or multinucleate. It is possible that this distinction in wall structure of primary spores may assume a greater significance as more entomophthoralean fungi are found and described.

Among the characters related to primary spores in the Entomophthorales, then, generic value should be accorded to at least the number and nature of the nuclei in the spore. The shape and uni- or bitunicate nature of the primary spore should always be noted. However, while various states of these characters may coincide perfectly with the generic limits defined by other criteria, no reason is apparent at this time why

either of them should be accorded generic value equal to nuclear number and morphology.

## PRIMARY SPOROPOHORES

### Morphology and Branching

The branching of sporophores was one of the first criteria used to divide the entomopathogenic Entomophthorales into two genera (Brefeld 1873, 1877; Nowakowski 1883), and has continued to be one of the leading characters used by Batko (1964a-c, 1974; Batko and Weiser 1965) to construct a contemporary classification. The historical significance of sporophore branching as a character with generic importance demands careful review of both the reasons for and the effects of its de-emphasis by Remaudière and Keller (1980). It is necessary first to examine the systematic distribution of simple and branched sporophores and then to evaluate the exceptions to the normal state in each genus.

For any practical purpose, the sporophores are always simple in *Entomophthora* (although in *E. culicis* several sporophores may arise from each hyphal body, thus giving an impression of branching), *Massospora*, *Triplosporium*, and *Completozia* Lohde, a parasite of fern prothallia. *Tabanomyces* Couch et al. (1979; from tabanid fly larvae) and *Meristacrum* Drechsler (1940; from nematodes) produce an upright, unbranched sporogenous hypha which becomes septate and produces forcibly discharged spores on lateral (or terminal) papillae. *Ballocephala* Drechsler (1951; from tardigrades) also produces an upright, unbranched sporogenous hypha on which small lateral cells are formed from which the spores are budded and forcibly discharged. Sporophores in species of *Ancylistes* Pfister (Berdan 1938; from desmid algae) and *Basidiobolus* (Drechsler 1964) may occasionally branch; those of the 27 *Conidiobolus* species recognized by King (1976b, 1977) also may, on rare occasion, be bi- or trifurcate (King and Humber 1981). Among the species transferred to *Conidiobolus* by Remaudière and Keller (1980) the sporophores of *Empusa apiculata* Thaxter and *E. major* Thaxter may show some branching on rare occasion (see Thaxter 1888, Fig. 63), but are almost always simple. There is no significant branching in any *Entomophaga* species.

All species of *Zoophthora* s.str. (Remaudière and Hennebert 1980; Keller 1980; Ben-Ze'ev and Kenneth 1981a-b) have digitately branched sporophores with the possible exception of *Z. crassitunicata* Keller (1980) in which the sporophores appear in histological sections to be mostly simple.\* All but three *Erynia*

species (Remaudière and Hennebert 1980; Remaudière and Keller 1980); Kramer 1981) have digitately branched sporophores.

Within the bounds of the usual biological variation it is not surprising that some species with simple sporophores occasionally have a small number of sporophores that show a low order of branching. Whatever branching occurs in these genera is usually basal rather than apical as in *Zoophthora* and *Erynia* (where the apical, digitate branching pattern usually results in the formation of a tightly interwoven hymenium). The infrequent occurrences of branched sporophores in genera characterized by simple sporophores or of occasional simple sporophores in genera characterized by branched sporophores in no way indicates that this character should be de-emphasized or discarded.

The sporophores of *Zoophthora* and *Erynia* (apart from the three exceptions discussed below) do exhibit a variable degree of branching. Unbranched sporophores might occasionally be found on specimens from this large group of species in which nearly all sporophores are digitately branched. Similarly, species such as *Z. crassitunicata* may exist in which (digitately?) branched sporophores are less numerous than simple ones, but the affinities of these species with *Zoophthora* or *Erynia* will always be apparent from the natures of those branched sporophores, their primary spores, and other associated characters.

The natural degree of plasticity in the branching of sporophores in species of *Zoophthora* and *Erynia* does not imply, however, any real probability of finding species such as *Z. radicans* (Bref.) Batko (= *Entomophthora sphaerosperma* Fres.) of *Erynia neoaphidis* Rem. & Henneb. (= *Entomophthora aphidis* Hoffman sensu Thaxter) which form obviously digitate sporophores to yield an exceptional specimen in which a majority (much less all) of the sporophores are simple. It is even less feasible that a strain of such a species exists which produces branched sporophores when infecting most hosts but which forms only simple ones on one or more other hosts.

The uniform occurrence of digitate sporophores in *Zoophthora* sensu Remaudière & Hennebert – including those (digitately?) branched sporophores of *Z. crassitunicata* – leaves only the two species of *Strongwellsea* and *E. caroliniana*, the only three exceptional species placed in *Erynia* by Remaudière and Keller (1980) of sporophore branching as a generic criterion.

It is important to examine why Remaudière and Keller (1980) supposed that a significant probability exists for finding branched sporophores in the two *Strongwellsea* species. It is these fungi which seem to form the basis for their de-emphasis of sporophore branching. The statement that branched sporophores are "not always present" in *Strongwellsea*, their primary justification for their opinion, seems to draw solely

\* Keller's (1980) characterization of the sporophores of *Z. crassitunicata* as mostly simple seems to be based entirely on histological sections and should, therefore, be accepted as provisional until the publication of micrographs of carefully dissected pieces of hymenium showing whole sporophores of this species.

upon Strong et al. (1960) who note that the sporophores are "rarely branched" but who also admit the difficulty of tracing complete structures in their prepared slides. Batko and Weiser (1965) used some of these slides and correctly described the sporophores of *S. castrans* Batko & Weiser (1965) to be unbranched; they neither illustrated nor noted any exception to this simple state. Humber (1975, 1976) also found the sporophores of *S. magna* Humber to be *always* simple, and included this characterization in the emended generic description of *Strongwellsea*. The uniformly unbranched nature of sporophores in this genus reflects the means by which additional sporogenous hyphae interpolate themselves into and thus continually expand the surface area of the fungal ball in the host fly's abdomen (Humber 1975).

It has already been noted that *E. caroliniana* (with multinucleate and unitunicate spores, simple sporophores, and lack of rhizoids or cystidia) shares no major characteristics with *Erynia* species except for spore shape; the simple sporophores of this species do not constitute any significant exception to the common state of digitately branched sporophores in *Erynia*. However, some minor degree of branching in *E. caroliniana* sporophores may occur *inside* the host body, but the emergent sporophores are uniformly unbranched on the surface of affected crane flies (Thaxter 1888). Both Giard (1888, as *E. arenocetona* Giard) and Keller (1978) also find the sporophores of *E. caroliniana* to be simple.

Several aspects of sporophore morphology – the presence and pattern of branching and the overall shape of the sporogenous cell or sporophore apex – should be considered to be important, but generic significance has usually been placed only on whether a sporophore is simple or branched. The sporophores of all *Entomophthora* species (in the strict sense) are markedly swollen and clavate below the spore, but this is the only genus in which this character is uniformly present. There is a less consistent tendency to produce clavate sporophores in species of *Zoophthora* or *Erynia*. In most other species and genera of the order, the apical portion of the sporophore is relatively cylindrical with only a slight tendency to become clavate.

Srinivasan et al. (1964) proposed to separate *Conidiobolus* from *Entomophthora* on the basis of sporophore shape. They suggested that *Conidiobolus* species have micronemous (hypha-like, indeterminate) sporophores while those of *Entomophthora* are macronemous (thicker than and distinctly differentiated from the vegetative hyphae, and of determinate length). This criterion was devised to separate *Conidiobolus* species from *Entomophthora muscae* (the type of its genus) and paid little attention to the many other species in *Entomophthora sensu lato*. In the more modern taxonomic systems considered here, the criterion proposed by Srinivasan et al. (1964) is

applicable only to the few species of *Entomophthora* s.str. (Remaudière and Keller 1980); it is also untenable, however, since *Conidiobolus adiaeretus* Drechsler (see King and Humber 1981, Fig. 4f) and some other *Conidiobolus* species have markedly differentiated (macronemous) sporophores.

Several genera can, in fact, be distinguished in part by the exact morphology of the sporophore. *Basidiobolus* species have a more or less prominent swelling of the sporophore immediately below the spore, and the sporophore is markedly narrowed at the point where the spore is attached. Species of *Meristacrum*, *Tabanomyces*, and *Ballocephala* from several spores on each unbranched erect sporogenous hypha. The mode of spore production in *Meristacrum* and *Tabanomyces* is identical, and these genera are here considered to be synonymous (see below). The spores of *Ballocephala* are produced on globose to elongate lateral cells produced sympodially at the growing apex of the sporogenous hypha (Drechsler 1951; Richardson 1970; Pohlad and Bernard 1978). Three species placed in *Conidiobolus* by Remaudière and Keller (1980) – *E. apiculata*, *E. major*, and *E. papillata* Thaxter – differ from all other species of that genus (in the sense of King 1976b, 1977) by having an extended and neck-like narrowing of the sporophore apex; this morphological difference suggests that all characters of these fungi should be compared carefully with those of *Conidiobolus* before accepting their placement in this particular genus.

#### Taxonomic Significance of Primary Sporophores

The presence of branched or simple sporophores was regarded as a primary generic character in the Batko classification but was de-emphasized (and effectively rejected) in the Remaudière classification, seemingly to justify the synonymy of *Strongwellsea* in which the sporophores are *always* simple) with *Erynia* (in which the usual case is for sporophores to be digitately branched). This de-emphasis and the elevation of spore morphology to a primary generic character by Remaudière and Keller (1980) further confused the classification by placing *E. caroliniana* (with simple sporophores) in *Erynia* and *Entomophthora carpentieri* Giard (apparently with branched sporophores, see below) in *Conidiobolus*.

As has been advocated by Ben-Ze'ev and Kenneth (1981a) sporophore branching should be reinstated as a primary generic character. This restoration sweeps aside the apparent misclassifications of the Remaudière scheme by returning *Strongwellsea* to its rightful status as a separate genus (Humber 1975, 1976, 1981a) and leaving the placements of *E. caroliniana* and *E. carpentieri* undetermined and in need of further study.

## MODE OF FORCIBLE DISCHARGE OF PRIMARY SPORES

Three distinct mechanisms for the forcible discharge of primary spores are known from the Entomophthorales. Two of these mechanisms appear to be entirely restricted to single clearly defined genera while the predominant mechanism operates in nearly all the remaining genera. Primary spores are forcibly discharged in all generally recognized genera of this order except for *Massospora*, in which spores and hyphal bodies are passively dispersed from the disintegrating abdomens of the living host cicadas. All of the diverse types of secondary spores are also forcibly discharged except for those capillisporous (see below) produced on long, narrow capillary sporophores.

All species of *Entomophthora* sensu stricto (Remaudière and Keller 1980) have campanulate and apiculate primary spores which are forcibly discharged on a stream of cytoplasm and vacuolar sap squirted from the sporophore as the wall layers(s) securing the primary spore to the sporophore break. The spore itself has a flat or slightly convex base rather than a distinct papilla, and appears to play no active role in the discharge process. Spores discharged in this manner are accompanied throughout their trajectories by a voluminous drop of the protoplasm which serves to affix the spore to the surface on which it lands, and forms a characteristic radially striate corona around the primary spore upon drying.

The primary spores of all species of the non-entomogenous genus *Basidiobolus* are borne on sporophores with a pronounced swelling immediately below the primary spore. Discharge occurs with a circumscissile rupture of the lower portion of the swelling; the spore and swollen upper portion of the sporophore fly away as the hydrostatic pressure in the upper portion of the sporophore is released backwards like a small rocket (Ingold 1934). Ingold's rocket analogy is strengthened by the fact that it is often possible to obtain the firing of a "second stage" as the small conical projection of the *Basidiobolus* sporophore into the spore itself may evert during flight, thus giving a slight push against the sporophore fragment and assuring a somewhat longer trajectory for the spore than for the sporophore fragment from which the spore becomes detached.

With the exception of *Massospora*, all other genera of the Entomophthorales apparently have primary spores discharged by the eversion of the spore's papilla against the sporophore (Gallaud 1905; Couch 1939). This mechanism depends upon the establishment of high turgor pressures in both the sporophore and spore prior to the rounding off of the turgid cell (Ingold 1971). The sporophore wall breaks at the point of spore attachment, and the spore papilla everts rapidly from its original position pointed into the spore and pushes the spore away from the turgid sporophore. The shock of this eversion against the pressurized

water column of the sporophore sometimes causes a rupture of the sporophore tip and exudation of a water droplet at the time of discharge (e.g., Page and Humber 1973).

Despite the lack of detailed understandings of these discharge mechanisms and of the involvements of the various wall layers of the sporophores and spores, it is apparent that spore discharge is a major event in the life history of an entomophthoralean fungus requiring the expression of a considerable portion of the genome. In view of the significance of this process for the fungus and the restricted distributions of the three diverse mechanisms, it is appropriate to recognize the mode of forcible discharge of primary spores as having taxonomic value at least on the generic level.

## MODE OF SECONDARY SPORULATION

The general ability of entomophthoralean fungi to produce one or more types of secondary spores from primary spores is one of the most unusual features of this group of fungi. Among other fungal taxa, the Sporobolomycetaceae (heterobasidiomycetous "mirror" yeasts), many ascomycetes whose ascospores may bud repeatedly in the ascus, and some other entomopathogens such as a few species of *Septobasidium* Patouillard (Couch 1938) and the hyphomycetous genera *Muiozone* Thaxter (1920) and *Termitariopsis* Blackwell, Samson & Kimbrough (1980) may produce secondary spores. In none of these groups, however, has secondary sporulation assumed such a significant role as in the Entomophthorales. It is becoming increasingly apparent, for instance, that the secondary spore types are equally as infective as primary spores or that primary spores serve as dispersive units while the secondary spores produced from them may even be the major infective units (Carner 1976; Carner and Canerday 1968; Kramer 1980; Nemoto and Aoki 1975; Nemoto et al. 1979; Selhime and Muma 1966; Tsintsadze and Vartapetov 1976; Wilding 1970).

The morphological diversity (Batko 1974; King and Humber 1981) and biological importance of these spore types suggests that they might have taxonomic value above the specific level. In fact, two types of secondary spore forms – microspores and capillisporous – have formed the bases for taxonomic divisions at subgeneric or generic levels in the Entomophthorales.

Microspores are produced when the primary spores of some *Conidiobolus* and *Basidiobolus* species produce a few to several dozen small secondary spores which are forcibly discharged and act individually as asexual propagules. The formation of microspores by *C. coronatus* (Costantin) Batko was first used to distinguish the genus *Delacroixia* Sacc. & Sydow; Tyrrell and MacLeod (1972) proposed that *Delacroixia* should be regarded as a subgenus of *Conidiobolus* to

accommodate all species of that genus capable of forming microspores.

Capillispores (despite a profusion of names which have been applied to them) are passively detached spores produced singly atop a thin and elongate (capillary) sporophore. Batko (1966) proposed to divide *Zoophthora* among four subgenera with all species producing almond-shaped to elongate secondary spores on capillary sporophores to be restricted to the subgenus *Zoophthora* Batko. Remaudière and Hennebert (1980) restricted the genus *Zoophthora* to those species producing capillaries and capillispores and transferred all species remaining in the other three subgenera proposed by Batko (1966) to *Erynia* Nowakowski. In addition to their presence in all species of *Zoophthora* subg. *Zoophthora* sensu Ben-Ze'ev & Kenneth (1981a) or *Zoophthora* Batko sensu Remaudière & Hennebert (1980), capillispores are produced by all species of *Triplosporium* sensu Batko (but not *Neozygites* sensu Remaudière & Keller 1980), *Meristacrum* (including *Tabanomyces*; see below), and some species of *Conidiobolus* and *Basidiobolus*. Batko (1974) considers the presence of capillary sporophores to reflect an ancestral character of the Entomophthorales and rejects the possibility of their separate origins in these genera by evolutionary convergence.

If the Remaudière classification were perfectly even-handed in its adoption of a generic criterion based on capillispores, then *Entomophthora turbinata* Kenneth (1977), which produces no capillispores, should not have been included in *Neozygites* by Remaudière and Keller (1980), and both *Conidiobolus* and *Basidiobolus* should have been split. It is curious that Remaudière and his colleagues apparently do not accept the parallel notion of splitting *Conidiobolus* because of the presence of microspores in some species of this genus.

Ben-Ze'ev and Kenneth (1981a) correctly reject the generic emphasis placed on capillispores by Remaudière and Hennebert (1980), and propose to use microspores and capillispores as a character with subgeneric validity as did Batko (1966) and Tyrrell and MacLeod (1972) before them. However, it is neither advisable nor even possible to apply criteria based on specialized secondary spore forms to all genera in which they might occur since *Basidiobolus microsporus* forms both microspores and capillispores (Benjamin 1962).

## RHIZOIDS

### Historical Perspective and Taxonomic Distribution

Batko's (1964a-c, 1974) placement of generic value on the presence of rhizoids has been one of the most troublesome aspects of his classification of the entomopathogenic Entomophthorales. His adoption of this criterion narrowed his circumscription of *Ento-*

*mophthora* s.str. and led him to disperse the few species now regarded to belong in this genus (Remaudière and Keller 1980) among two genera. This misplaced emphasis also led him to propose *Culicicola* and *Entomophaga*, separated by the respective presence or absence of rhizoids, genera whose validity and circumscriptions have remained suspect due to the general controversy over the validity of rhizoids and to Batko's inability to provide a definitive separation of *Conidiobolus* from *Entomophthora* sensu lato.

Humber et al. (1977) re-evaluated and rejected the significance of rhizoids described for *Entomophthora virulenta* Hall & Dunn (1957; = *Conidiobolus thromboides* Drechsler; see Latgé et al. 1980). The rhizoids described for this fungus are now regarded to have belonged to another entomophthoralean fungus affecting the same aphids but which produced no spores or whose spores were undetected.

Remaudière and Keller (1980) and King and Humber (1981) independently discuss those species which usually form rhizoids but occasionally fail to do so. Note that all these exceptions represent the absence of (or failure to find) rhizoids in species normally producing them; they never involve observations of rhizoids in species not known to form them. Remaudière and Keller (1980) believe that no weight should be put on the presence of rhizoids because their presence "in certain species" is not always constant. On the contrary, King and Humber (1981) regard the presence of rhizoids to be taxonomically significant while their absence is not a dependable criterion.\* Ben-Ze'ev and Kenneth (1981a) regard the presence of rhizoids to be taxonomically significant at the subgeneric level. Brobyn and Wilding (1977), in the most thorough consideration of the development of rhizoids and cystidia yet published, uphold the presence of rhizoids to be a taxonomically significant character.

It is necessary to consider the general situation to know if any confidence should be placed on the presence or absence of rhizoids as a taxonomic character. Even though no absolute scale is available to quantify one's level of confidence, it is necessary to weigh how well a species is known: How many times has it been found? In how many sites? From how many hosts? Are rhizoids always present or always absent? An examination of the taxonomic distribution of rhizoids among the entomophthoralean species reveals that many have never been found with them present. In only a few species, particularly *Zoophthora radicans* (= *Entomophthora sphaerosperma*), is the presence of rhizoids usual but inconstant. The absence of rhizoids might be explained in some instances by improper handling of specimens, but it is

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\* Editorial changes of the text unintentionally altered this opinion to state that the absence of rhizoids is not taxonomically significant.

also possible that their formation on some insects might be a function of that particular host (Remaudière and Keller 1980; King and Humber 1981).

**Genera without rhizoids.** Rhizoids are completely absent or have not been found in several genera. These include *Triplosporium* (at least 7 species), *Entomophaga* (at least 4 species although the circumscription of this genus remains in dispute), *Tabanomyces* (1 species; = *Meristacrum*), *Massospora* (13 species), and *Strongwellsea* (2 species). None of the non-entomopathogenic genera – *Basidiobolus*, *Ancylistes*, *Completozia*, *Meristacrum*, and *Ballocephala* – produce rhizoids.

***Entomophthora* (sensu stricto).** Two of the six species of *Entomophthora* s.str. (Remaudière and Keller 1980) produce rhizoids. This is the least constant appearance of this structure in any entomophthoralean genus. It can be said with assurance that rhizoids do not occur in the type species, *E. muscae*, since this is the most frequently observed of all species in the order. Both *E. culicis* (Braun) Fres. and *E. planchoni-ana* Cornu produce abundant rhizoids on hosts bearing forcibly discharged primary spores, but these anchoring structures may not occur when resting spores are formed. Both species are cosmopolitan in distribution and affect a wide variety of dipterans and aphids (respectively). All of the remaining species – *E. weberi* Lakon ex Samson (a little known species from a neuropteran), *E. thripidum* Samson & al. (1979; known only from thrips in Dutch greenhouses), and *E. erupta* (Dustan) Hall (which is known from several North American and European sites and hosts) produce no rhizoids and disperse their primary spores from living, mobile hosts. The same behavior occurs in the species of *Massospora* and *Strongwellsea*.

***Conidiobolus*.** Among the 27 morphologically homogeneous species recognized by King (1976a-b, 1977), none is known to produce rhizoids; very few *Conidiobolus* species are currently known to be entomopathogenic. Remaudière and Keller (1980) transferred most entomophthoroid species with round to pyriform spores from *Entomophaga* (which was circumscribed to exclude species with rhizoids) and those species from *Culicicola* with round spores to *Conidiobolus*. All other *Culicicola* species have campanulate spores and were restored to *Entomophthora* by Remaudière and Keller (1980).

Among the 38 *Conidiobolus* species listed by Remaudière and Keller (1980), four produce rhizoids: *Empusa apiculata* Thax., *E. major* Thax., *E. papillata* Thax., and *Entomophthora carpentieri* Giard (1888). The first three species were placed in *Culicicola* by Batko (1964c-d) because of their rhizoids and multinucleate round spores borne on simple (or occasionally branched) sporophores. Their pronounced similarity to *Conidiobolus* species belies two obviously morphological differences from all other *Conidiobolus* species:

the constant formation of a few stout rhizoids ending in strongly differentiated holdfasts, and the nearly cylindrical, collar-like narrowing of the sporophore apex. Close study of these species will be required to verify if they actually belong in *Conidiobolus* as suspected by King (1976b) and affirmed by Remaudière and Keller (1980).

The only morphological character of *Entomophthora carpentieri* Giard (1888) fitting *Conidiobolus* is the round primary spore supposed by Turian (1957) to belong to this species. Several other characters of this fungus – the presence of rhizoids, uninucleate spores on branched sporophores (Turian 1957), and cystidia (Petch 1944, if Petch's identification is correct) – correspond exactly with those of *Erynia* or *Zoophthora* in the Remaudière classification. *Entomophthora coleopterorum* (Petch 1944) has narrowly oval rather than round primary spores; the mode of rhizoidal attachment is identical with that of *E. carpentieri*, a species which Giard (1888) characterized only by the distinctive nature of its rhizoidal attachment. In a situation resembling that described above for the "rhizoids" of *C. thromboides*, one cannot discard the possibility that Turian (1957) may have observed the round spores of a secondarily invasive *Conidiobolus* species on a beetle which was already infected by *E. carpentieri* which was able to form its very characteristic rhizoids but no spores. No cytological staining or photomicrographic evidence supported Turian's contention that these round spores were uninucleate; it is possible that he mistook a central oil droplet or vacuole for a nucleus. The discrepancies between the Giard, Petch, and Turian concepts of *E. carpentieri* can be reconciled only after examining any existing herbarium material of these collections. In any event, however, the entire habit of this species refers it to *Erynia* or *Zoophthora* rather than to *Conidiobolus*.

***Zoophthora*.** The presence of rhizoids is strongly correlated with branched sporophores bearing uninucleate, bitunicate primary spores – the species of *Zoophthora* Batko or *Erynia* Nowakowski. All eight *Zoophthora* species recognized by Remaudière and Hennebert (1980) form rhizoids. By including four newly described species, *Z. lanceolata* Keller (1980), *Z. crassitunicata* Keller (1980), *Z. orientalis* Ben-Ze'ev & Kenneth (1981a), and *Z. petchii* Ben-Ze'ev & Kenneth (1981b), the total rises to at least 11 or 12 species known to produce rhizoids. Ben-Ze'ev and Kenneth (1981a) remain uncertain if rhizoids were present on the few specimens of *Z. orientalis* available to them.

***Erynia*.** Among the species listed by Remaudière and Hennebert (1980) and Remaudière and Keller (1980), some 21 of 25 *Erynia* species (excluding those of *Strongwellsea*) produce rhizoids. Together with *Ery-*

*nia delphacis* (Hori) Humber\*, *E. delpiniana* (Cavara) Humber\*\*, and *E. ithacensis* Kramer (1981), the total incidence of rhizoids rises to 22 of 28 species. The absence of rhizoids in the six *Erynia* species discussed below if not considered to overturn the value of rhizoids as a secondary generic character although *E. delphacis* and *E. delpiniana* constitute the most notable of these exceptions.

Thaxter (1888) suspected that rhizoids were present on *Empusa virescens* Thax., but could not be certain since he was not the original collector of the material which he later used to describe this species.

*Erynia aquatica* (Anderson & Anagnostakis) Humber\*\*\* is known only from mosquito larvae and pupae floating on the surface of temporary pools in the northeastern United States. This floating habit of the stricken hosts maintains their position for favorable transmission of the fungus without any "need" for rhizoids. This species is not known well enough, however, to say whether rhizoids might not form, for example on individuals trapped at the receding edges of rapidly drying pools.

Ben-Ze'ev and Kenneth (1979) report cystidia but no rhizoids from *Zoophthora erinacea* Ben-Ze'ev & Kenneth, but note the similarity of rhizoids and cystidia of species referable to *Zoophthora* subg. *Erynia* Batko (1966). They muse on the possible inter-relatedness of cystidia and rhizoids as did Thaxter (1888) and Gustafsson (1965). However, Brobyn and Wilding (1977) provide effective evidence that the functions and course of development of these structures are unrelated in species where they both occur together; it must be assumed, then, the rhizoids and cystidia are under completely separate genetic controls.

*Empusa caroliniana*, with its multinucleate unitunicate spores borne on simple sporophores and lack of cystidia or rhizoids, is once again shown to stand apart from *Erynia* in all major characters except for the shape of its primary spores. The correct generic assignment for this fungus remains problematic, although its spore karyology, sporophore morphology, and mode of spore discharge more closely resemble the species of *Entomophaga* than any other genus

recognized by Batko, Remaudière, or Ben-Ze'ev/Kenneth classifications.

*Erynia delpiniana* and *Erynia delphacis* may be true exceptions to the usually rhizoidal state of fungi in this genus. Cavara (1899b) is fastidious in his observations of the small, uninucleate, bitunicate primary spores, secondary spores like the primaries, digitately branched sporophores, and very large and occasionally branched cystidia of *E. delpiniana*. However, he never specifically mentions any hyphae affixing the affected flies to the damp piers of a bridge over a small stream. The flies were completely covered under a dense, dirty-white to straw-colored hymenium. This entire hymenial covering was easily broken away from the cadavers during attempts to detach the insects (Cavara 1899b). Except for the apparent absence of rhizoids all characters of this species match the characteristics used to describe *Zoophthora* subg. *Erynia* Batko (1966); all species in this subgenus have rhizoids and prominent cystidia. *Erynia delpiniana* is known only from Cavara's original collections, but it seems likely that examination of this material or any fresh collections would reveal the presence of rhizoids.

*Erynia delphacis* is a much more important exception to the presence of rhizoids than the other species considered above. This pathogen of leaf- and plant-hoppers in Asian rice paddies bears a strong morphological resemblance to *E. neoaphidis* Remaudière & Hennebert (= *Entomophthora aphidis* Hoffman sensu Thaxter) from aphids (Shimazu 1976, 1977; Remaudière and Hennebert 1980), but differs notably in the complete lack of rhizoids on any of its hosts, including two artificially infected aphid species, *Aphis gossypii* and *Macrosiphum akebiae* (Shimazu 1977, and personal communication). *Erynia delphacis* produces cystidia both on its host and in culture. Even though Hori (1906) illustrates and describes spherical, yellow-white resting spores 24-28 µm in diameter and containing many fatty granules, no such spores have been found in subsequent collections of this species in Japan, the Philippines, or Indonesia, nor are they formed in culture.

Remaudière and Hennebert (1980) consider the differences of growth *in vitro* between *E. delphacis* and *E. neoaphidis* to be insignificant and regard *E. delphacis* as a *nomen dubium*. However, a comparison of a Japanese strain (Shimazu F32; our RS 134) and several Philippine isolates from green leafhopper, *Nephotettix virescens*, with numerous cultures of *E. neoaphidis* isolated by Insect Pathology Research Unit personnel indicate that *E. delphacis* grows substantially more rapidly on a wider variety of media (nutritionally simple or complex), and sporulates a great deal more prolifically over a longer time than any culture of *E. neoaphidis* we have yet observed. Until more extensive studies can be made comparing these two species, it appears to be preferable to recognize *E.*

\* *Erynia delphacis* (Hori) Humber, **comb. nov.**, basionym: *Entomophthora delphacis* Hori, 1906, Entomol. Mag. (Tokyo) 3: 81.

\*\* *Erynia delpiniana* (Cavara) Humber, **comb. nov.**, basionym: *Entomophthora delpiniana* Cavara, 1899, Nuov. Giorn. Bot. Ital. (N. Ser.) 6: 422.

\*\*\* *Erynia aquatica* (Anderson & Ringo ex Anderson & Anagnostakis) Humber, **comb. nov.**, basionym: *Entomophthora aquatica* Anderson & Ringo ex Anderson & Anagnostakis, 1980, Mycotaxon 10, 350, **non** *Entomophthora aquatica* Anderson & Ringo, 1969, J. Invertebr. Pathol. 13, 386 (which was invalidly published without a designated type). The combination *Erynia aquatica* (Anderson & Ringo) Remaudière & Hennebert, 1980, Mycotaxon 11, 301, is also invalid according to Article 45 of the International Code of Botanical Nomenclature.

*delphacis* as a legitimate species of *Erynia*. It should also be noted that if later studies indicate the synonymy of *E. delphacis* and *E. neoaphidis*, the International Code of Botanical Nomenclature would require that *E. delphacis* (Hori) Humber must be the correct name for the fungus still generally known as *Entomophthora aphidis* Hoffm. (sensu Thaxter).

Much more pertinent information is needed about the morphology of rhizoids than the perfunctory characterization found in many descriptions that rhizoids are merely "present." As noted by King and Humber (1981), complete descriptive information about rhizoids should include their points of emergence, dimensions (particularly thickness), abundance (few or many?), relative spatial distribution (isolated, aggregated into one or more pseudorhizomorphs, or forming a veil?), and the morphology of any terminal holdfast apparatus. Thaxter (1888), Gustafsson (1965), Ben-Ze'ev and Uziel (1979), and Ben-Ze'ev and Kenneth (1981a-b) are exemplary in the clarity of their illustrations and characterizations of rhizoidal morphology. This descriptive information can be useful for detecting dual infections involving rhizoidal and non-rhizoidal species since Brobyn and Wilding (1977) affirm that the morphologies of rhizoids appear to be species specific.

At least three morphological classes of rhizoids can be recognized: Most rhizoidal species have either (1) numerous individual rhizoids which are relatively little differentiated from vegetative hyphae and may or may not have any terminal holdfast differentiation, (2) numerous hypha-like rhizoids aggregated into one or more columnar pseudorhizomorphs, or (3) relatively few, thick rhizoids with strongly differentiated (usually discoid) terminal holdfasts. No comprehensive study of the development and morphology of rhizoids or of their taxonomic distribution has yet been undertaken. The thorough studies of Brobyn and Wilding (1977) were limited to a few species affecting aphids. In view of the observations presented here, however, such a comprehensive study would be exceptionally useful.

#### Rhizoids as a Taxonomic Character

The Batko classification adopted the widely accepted contention of Nowakowski (1883) that the presence of rhizoids is a major generic character. This criterion, superimposed on those of nuclear number in primary spores and the branching of sporophores had effects which have been regarded as unacceptable and have prevented the general acceptance of the Batko classification (Remaudière and Keller 1980; King and Humber 1981). The Remaudière classification, in turn, rejected the significance of the presence of rhizoids. It seems certain, however, that the absence of rhizoids in many genera is significant while their presence in others is an important secondary character.

Regardless of the level (if any) at which one may accept the taxonomic importance of rhizoids, species presenting exceptions to the normal rhizoidal or non-rhizoidal condition of their genus should be examined carefully. The classifications of *Strongwellsea castrans*, *S. magna*, and *E. caroliniana* in *Erynia* and of *E. apiculata*, *E. major*, *E. papillata*, and *E. carpentieri* in *Conidiobolus* are important examples of possible misclassifications pointed out by the lack or possession of rhizoids.

#### CYSTIDIA

Cystidia (pseudocystidia, paraphyses, or pseudoparaphyses for other authors) are short to long, spear-like to columnar structures of determinate growth that project above the level of the sporogenous hymenium. Until recently, little has been known of their function, but Brobyn and Wilding (1977) convincingly demonstrated that the cystidia of *Erynia neoaphidis* are penetration organs which rupture the host cuticle and aid the subsequent emergence of the sporophores. The emergence of cystidia before the sporophores also has been noted in *E. erinacea* (Kenneth 1977) and *E. ithacensis* (Kramer 1981). It seems likely that cystidia serve to perforate the host cuticle prior to hymenial formation in all species in which they occur; future investigators should be careful to note the order of emergence of cystidia and sporophores.

Remaudière and Keller (1980) indicate that, apart from *Erynia* and (less commonly) *Zoophthora*, the only other genus in which cystidia occurs is *Conidiobolus*. Among the 38 species of *Conidiobolus* accepted by Remaudière and Keller, the only ones ever mentioned to form cystidia are *C. obscurus*, *C. thromboides* (= *E. virulenta*), and *E. carpentieri* (as *E. coleopterorum* Petch 1944); as was noted above, the latter species almost certainly belongs in *Erynia* or *Zoophthora* rather than *Conidiobolus*.

The description of *E. virulenta* (= *C. thromboides*; see Latgé *et al.* 1980) states that "cystidia occur rarely" (Hall and Dunn 1957). However, as explained above for the "rhizoids" of this species, no subsequent treatment of it reports finding either rhizoids or cystidia. The "cystidia" of *C. thromboides* might have been those of another entomophthoralean fungus which produced both rhizoids and cystidia (see Humber *et al.* 1977). Hall and Dunn (1957) might also have interpreted vegetative or sporogenous hyphae whose indeterminate growth occasionally far exceeds the general level of the hymenium to be cystidia.

Remaudière *et al.* (1979) report the formation of long cystidia by *C. obscurus* on aphid cadavers placed in a humid environment. I have observed similar growth of long hyphae above the hymenium of aphids affected by *C. thromboides* but always find these structures to be undifferentiated vegetative

hyphae which should not be regarded as cystidia by the definition given above.

The listing of *Lamia* (= *Entomophthora*) *culicis* in a chart by Nowakowski (1883) as having cystidia present and rhizoids absent was corrected by Batko (1964c) as being a typographical transposition not corresponding with the Polish text which correctly notes this species to have rhizoids but no cystidia. The genus most closely related to *Zoophthora* and *Erynia* must be *Strongwellsea*, with its uninucleate and bitunicate primary spores borne on simple rather than branched sporophores (Batko and Weiser 1965; Humber 1976). Remaudière and Keller (1980) consider the lack of rhizoids and cystidia to be insignificant criteria and use this information to explain their submergence of *Strongwellsea* into *Erynia* (see Humber 1981a). In fact, if cystidia function only for cuticular penetration, it is not surprising that *Strongwellsea* species for no cystidia. These fungi produce no hymenial structures outside the host's body. The abdominal hole (the only cuticular rupture caused by the fungus) begins with a point perforation of the pleuron and enlarges progressively. This point initiation of the rupture appears to be involved with the hypertrophic reaction of the host cuticle which leads, in turn, to the extensive lining of the lower portion of the fungal ball by the host's cuticle. It seems clear that some mechanism completely distinct from that of cystidial penetration is involved in the formation by *Strongwellsea* of an abdominal hole (see Humber 1975, 1976).

In view of the above discussion, it is probably correct to say that the only species in which cystidia (as determinate organs for cuticular penetration) occur are those with uninucleate, bitunicate primary spores borne on branched sporophores and classifiable in *Erynia* or (less commonly) *Zoophthora* sensu Remaudière & Hennebert.

Unlike for rhizoids, the presence or absence of cystidia has never been used to separate genera although the Batko classification recognized their value as a supporting character strongly correlated with the occurrence of uninucleate, bitunicate primary spores and branched sporophores. As with the other characters discussed here, exceptions to the usual state of the character in a genus should indicate that a close inspection of the correspondences of all characters of the suspect species with those of other species in the genus is in order. The presence of cystidia in *E. coleopterorum* (Petch 1944), a presumptive synonym of *Conidiobolus carpentieri* (Giard) Remaudière & Keller, offers a significant example of this principle.

## RESTING SPORES

### Zygospores or Azygospores:

#### a Morphological/Cytological Dilemma

I applaud the de-emphasis by Remaudière and Keller (1980) of the distinction between zygospores and azygospores. They note correctly that resting spores are not known (or are not formed) in a series of species, and that the mode of their formation has not been observed in a large proportion of species that do produce resting spores. Both types of development may occur in closely related species or even within a single species (MacLeod and Müller-Kögler 1973; Humber 1976; Remaudière and Keller 1980; King and Humber 1981).

This developmental distinction has been used only twice as a taxonomic character of generic importance. The peculiar mode of conjugation of (amoeboid?) hyphal bodies or gametangia of *Empusa fresenii* Nowakowski 1883 (= *Neozygites aphidis* Witlaczil 1885) and the resultant jet-black, ovoid zygospores formed at the point of conjugation prompted Thaxter (1888) to set this and related species apart in *Empusa* subg. *Triplosporium*. This taxon was later raised to generic status by Batko (1964b), who was apparently unaware of the nomenclatural priority of the generic name *Neozygites* Witlaczil (see Humber *et al.* 1981). King (1976b) notes that the double-walled resting spores formed by the species of *Conidiobolus* are anisogamous zygospores. Other types of conidioboloid resting spores such as chlamydo-spores and villose spores are distinguished from zygospores (or azygospores) by their single rather than double wall structures.

Couch (1939) notes that the zygospores of *Conidiobolus* species are formed in the larger of two gametangia (and are almost always produced in the axis of the parental hyphae) while those of *Entomophthora* (sensu lato) are produced in lateral buds from the conjugating hyphae or hyphal bodies. Very few species of *Entomophthora* s.l. produce their zygospores in the hyphal axis: *E. virulenta* (= *C. thromboides*), *E. obscura* Hall & Dunn [= *E. ignobilis* Hall & Dunn = *E. thaxteriana* (Petch) Hall & Bell; see below], and *Empusa dipterigena* Thaxter. The former two species are now recognized as species of *Conidiobolus* by Remaudière and Keller (1980). With the apparent exception of *E. dipterigena*, whose zygosporogenesis is illustrated by Gustafsson (1965), the resting spores of other species of *Entomophthora* or its segregates are budded off from the parental hypha or hyphal body.

Zygospores, the bilayered, thick-walled spores derived from a conjugation of two gametangia, have been a well established phenomenon in the Entomophthorales since their first recognition (Brefeld 1873; Nowakowski, 1877, 1882, 1883; Thaxter 1888). Nearly every subsequent major dis-

cussion of these fungi has referred to zygospores as sexual spores and to azygospores as asexual spores (Schröter 1897; MacLeod 1956, 1963; Gustafsson 1965; Waterhouse 1973).

In general morphological terms, zygosporogenesis may be called a sexual process, in which case azygosporogenesis must be regarded as asexual. However, in their strict sense, the terms *zygospore* and *azygospore* refer only to the mode of development of a thick-walled spore in the Zygomycotina (Fitzpatrick 1930; Bessey 1950; Ainsworth 1961; Snell and Dick 1971; Alexopoulos and Mims 1979).

There is a hazard in thinking of zygospores as sexual and azygospores as asexual: One can be misled easily into performing an act of semantic sleight-of-hand by then expecting that the *genetic* events of a sexual life cycle, karyogamy and meiosis, necessarily occur in a "sexual" zygospore but cannot occur in an "asexual" azygospore. A related terminological confusion can occur when one refers to the resting spore as the sexual spore in the entomophthoralean life cycle as opposed to the forcibly discharged primary (or secondary) spores which are, in every sense, asexual spores.

Nuclear events in entomophthoralean resting spores – reductions in nuclear number, karyogamy, and meiosis – remain completely uninvestigated in all but a few species. This gap in our knowledge reflects a lack of fundamental studies on the life cycles in this group.

Some cytological studies have noted the progressive reduction of nuclear numbers to two or even a single nuclei in fully mature resting spores (Vuillemin 1900a-b; Krenner 1961; Latgé 1976; Couch et al. 1979; D. McCabe and B. Martinell, unpublished). Other investigators using different species have found no obvious change in the nuclear number from the time of formation to the fully mature, dormant state (Olive 1906; Riddle 1906; Goldstein 1929; Humber 1975). If the Entomophthorales follow patterns of karyogamy and meiosis similar to those in other Zygomycetes (Cutter 1942a-b), karyogamy should occur in the resting spore followed by meiosis in the spore just before or during its germination. No conclusive cytological evidence of nuclear fusions or of meiotic division has yet been found in entomophthoralean fungi despite the strong circumstantial evidence for their occurrences (*e.g.*, Couch et al. 1979). It must be hoped that synaptonemal complexes, a widely recognized ultrastructural marker for chromosomal synapsis, will be demonstrated at the time and site of the presumptive meiosis in one or more members of the Entomophthorales.

Data regarding changes in nuclear numbers presented by Latgé (1976) support the possibility that karyogamy and meiosis occur in the resting spores of *C. thromboides*, and that the vegetative nuclei of this species must be haploid. Albeit, Latgé feels that his

data are also compatible with gametangial meiosis of diploid nuclei such as occurs in the Oomycetes (see Alexopoulos and Mims 1979). Little ambiguity about the interpretation of the life cycle remains, however, in view of the results of electrophoretic analyses of isozyme patterns of several strains of *C. thromboides* and *Z. radicans* (May et al. 1979) and of *E. muscae*, *Massospora cicadina* Peck, and *Entomophaga grylli* (Fres.) Batko (Soper, May, and Martinell, in preparation). These results suggest that the vegetative nuclei of these species are haploid, although heterokaryosis may occur in some species since multiple alleles of some loci may be present in a mycelium.

There is no evidence yet of heterothallism within the Entomophthorales. All zygosporogenesis appears to be homothallic and leaves open at least two possible interpretations for the life cycles of these fungi: Those species whose zygospores or azygospores at some point contain only a single nucleus probably have autogamous (sexual) life cycles. Those species whose zygospores or azygospores undergo no reduction in nuclear numbers may have abandoned a sexual life cycle in favor of an apogamous (asexual) life which preserves the particular (successful) combination of characters in that species. No evidence now exists that parasexuality occurs in any species of the Entomophthorales.

The distinction between zygospores and azygospores cannot be retained as a character of taxonomic significance although the presence and nature of zygospores is a useful ancillary character in at least *Triplosporium* and *Conidiobolus*. The developmental origins of resting spores can be difficult to interpret correctly even if suitable material is available. Nuclear events in resting spores are of far greater importance for the fungus than whether conjugations precede sporogenesis. Karyogamy and meiosis, the critical genetic events in a sexual life cycle, apparently may occur in either zygospores or azygospores; similarly, other species may have apogamous life cycles regardless of whether their resting spores are zygospores or azygospores.

Indeed, the full details of entomophthoralean life cycles and their breeding strategies may yet prove to be useful taxonomic characters, but not enough is currently known of these processes to draw meaningful conclusions. There is no doubt, however, that this information is of absolutely vital importance for the practical development and manipulation of these fungi for use in the microbial control of their insect hosts.

#### Resting Spores: Problems and Interpretation of Taxonomic Traps

It appears that the genetic events of sexual reproduction might occur in either zygo- or azygospores, and that even species producing "sexual" zygospores might be apogamous. Several further caveats and an example serve to reinforce the rejection of any

taxonomic value which might be attached to the distinction between zygospores and azygospores (Remaudière and Keller 1980).

In most instances, authors simply state their observation that the resting spores of a given species are zygospores or azygospores and neither illustrate nor discuss the evidence supporting this conclusion. However, Sawyer (1931) notes the great difficulty in determining if conjugations occur in *Z. radicans*. Humber (1975) also notes difficulties with the resting spores of *Strongwellsea* species due to problems of interpreting the morphology of some hyphal bodies and to the evanescence of the emptied hyphal wall remnants which provide the only temporary evidence for the mode of formation of mature resting spores; determining the "pedigree" of these resting spores if dependent upon seeing them during a relatively narrow period during their development. Consequently, it seems unwise to place full confidence in any characterization of resting spores as zygo- or azygospores unless convincing documentation is also presented.

The difficulty in correctly applying these developmental designations casts doubt on whether any taxonomic decisions should be based solely on this distinction (even though it may have some utility for characterizing, but not separating, some genera as noted above). The practical effects of differing viewpoints of the value of this distinction can be seen in the following example:

In studies on the identities of aphid pathogenic species of the Entomophthorales, Humber (1978) noted that *Entomophthora ignobilis* Hall & Dunn should be used as the nomenclaturally valid name for *Entomophthora thaxteriana* (Petch) Hall & Bell, a species to which Hall and Bell (1963) considered *E. ignobilis* to be identical. *Empusa thaxteriana* Petch (1938) was proposed for a species misidentified by Thaxter (1888), and which was characterized as having azygospores. The resting spores of *E. ignobilis* are described by Hall and Dunn (1957) as zygospores. Humber (1978) considered the reported differences in the origins of resting spores to be so ambiguous as to be insignificant, an opinion which is reinforced

here. A later study by Remaudière *et al.* (1979) used morphological and biochemical data to demonstrate the synonymy of *E. thaxteriana* and *C. obscura* Hall & Dunn (1957) [= *Conidiobolus obscurus* (Hall & Dunn) Rem. & Keller], which was described as producing azygospores.

In fact, the differences between *E. thaxteriana*, *E. ignobilis*, and *E. obscura* had always been troublesome for diagnosticians. The only major described difference between *E. ignobilis* and *E. obscura* is that of zygospores versus azygospores, a difference considered here, by Humber (1978), and by Remaudière and Keller (1980) to be insignificant. A more consistent and simple approach to this taxonomic problem would have been to accept *E. obscura* to be the correct name by virtue of page priority over *E. ignobilis* rather than to regard the latter species as a *nomen dubium* merely because of the described difference in the origins of the resting spores in these two species (Remaudière *et al.* 1979).

If there is to be even-handed application of the accepted taxonomic criteria and acknowledgment of the net effects of rejecting other criteria which have been applied in the past, it is now necessary to recognize the following:

*Conidiobolus obscurus* (Hall & Dunn) Remaudière & Keller 1980, Mycotaxon 11, 331.

*Entomophthora obscura* Hall & Dunn 1957, Hilgardia 27, 162.

= *Entomophthora ignobilis* Hall & Dunn 1957, Hilgardia 27, 162.

= *Entomophthora planchoniana* Cornu sensu Thaxter 1888, Mem. Boston Soc. Nat. Hist. 4, 165, **non** sensu Petch 1938, Trans. Brit. Mycol. Soc. 21, 34.

*Empusa thaxteriana* Petch 1938, Trans. Brit. Mycol. Soc. 21, 34. **Name invalid without Latin diagnosis.**

[ *Entomophthora thaxteriana* (Petch) Hall & Bell 1963, J. Insect Pathol. 5, 186.]

[ *Entomophaga thaxteriana* (Petch) Batko 1964, Bull. Acad. Polon. Sér/ Sci. Biol. 12, 404.]

## CHOOSING A CORRECT CLASSIFICATION: TRIAL BY ANOMALY

One of the most severe tests for a classification system is to see how well it can handle the least typical species from the group being classified. Within the Entomophthorales, several seemingly anomalous generic assignments from both the Batko and Remaudière classifications are discussed here. However, in terms of testing the real capacities of these or any other classifications of these fungi, confirming the correlations of characters, or suggesting where primary taxonomic weights should be placed, the most

important fungus has not yet been considered. This is an undescribed species found by Balazy (1978) in Poland which was, obviously, not known at the time of the proposition of the Batko classification, and was not considered by the Remaudière classification.

This fungus affixes gnats (Diptera: Tendipedidae) by rhizoids to wet, decayed wood just above a stream surface in a deciduous forest in the Wielkopolski National Park, Poland. Its primary spores are slender and curved, 45-75 (82) x 8-10.5 µm, strongly tapered

apically and with a flattened, conical papilla. These characters almost exactly match those of *Erynia conica* (Thax.) Rem. & Henn., which has slender curved spores (of a size identical to Balazy's fungus) with bitunicate walls and single nuclei, borne on branched sporophores, and with cystidia and rhizoids present. Balazy's fungus is, however, clearly different from *E. conica* since its primary spores have unitunicate walls, contain 4-10 nuclei, and are borne on simple sporophores; cystidia are absent.

The Remaudière classification, with its primary emphasis on spore morphology, would place this species in *Erynia* even though that assignment would be impossible if one accepts the emended circumscription advocated here (excluding *Strongwellsea* and *E. caroliniana* from the genus by limiting it to species with uninucleate, bitunicate spores borne on branched sporophores and not producing capillispores). The Balazy fungus might be regarded as a species of *Culicicola* except that Batko (1974) apparently rejected *Culicicola* as a heterogeneous mixture of species now regarded to belong to *Entomophthora* s.str. or *Conidiobolus* (Remaudière and Keller 1980; King and Humber 1981). There is no basis for including the Balazy fungus in *Conidiobolus* according to the circumscriptions of this genus either stated or implied by Batko (1974), Remaudière and Keller (1980), here, or in Humber (1981b). It might be assumed that the Polish fungus could be allied to *E.*

*apiculata*, *E. major*, and *E. papillata* because of their multinucleate unitunicate spores, except that the nuclear morphology of the latter species corresponds to that of *Conidiobolus* species while that of the Polish fungus resembles *Entomophthora* and all of its segregates rather than *Conidiobolus* (see Humber 1981b). The generic system outlined by Ben-Ze'ev and Kenneth (1981a) would include this species in *Entomophthora* sensu stricto but the mode of spore discharge in the Balazy fungus is by the eversion of the papilla.

If, as suggested here, one rejects spore morphology as the primary generic criterion and accepts the restriction of *Erynia* noted above, and places generic value upon the karyology of primary spores, the morphology of the sporophore, and the mode of spore discharge, the only remaining sensible disposition for Balazy's fungus is in a new and (for now) monotypic genus allied to but differing from *Erynia*. It seems reasonable to assume that other entomophthoralean species may yet be found with characters like those of the one characterized by Balazy (1978).

It is imperative that this Polish fungus be fully characterized and more widely publicized. This fungus seems certain to provoke much useful discussion of the taxonomic criteria which should be accepted for entomophthoralean fungi, and of how much weight should be accorded to each of these criteria.

## MODERN VIEWS OF ENTOMOPATHOGENIC GENERA OF THE ENTOMOPHTHORALES

This study has examined the major morphological criteria used in entomophthoralean taxonomy, particularly as they are applied in the generic classification schemes proposed by Batko and by Remaudière and his colleagues. It is apparent from all of the above considerations that the application of only three characters provides an effective separation of species into morphologically and biologically homogeneous genera. These characters are the following:

1. The number and nature of nuclei in the primary spores.
2. The morphology of the sporophores (and the mode of sporogenesis).
3. The mode of discharge of primary spores.

Neither the morphology of primary spores, types of secondary spores formed, presence or absence of rhizoids, presence or absence of cystidia, nor the mode of formation of resting spores was found here to be important for delimiting genera in the Entomophthorales. Each of these characters, however, may have utility as secondary or supporting characters which do not in themselves determine the limits of genera. Some of these characters are appropriate for the circumscription of subgeneric taxa (Ben-Ze'ev and Kenneth 1981a).

It is appropriate to summarize the differences among the Batko and Remaudière classifications and that proposed preliminarily by Ben-Ze'ev and Kenneth (1981a), and to note those adjustments which seem to be necessary to circumscribe these genera still more accurately according to the criteria accepted here.

*Entomophthora Fresenius*. Batko's overestimation of the importance of rhizoids caused him to split a small and natural group of species (MacLeod *et al.* 1976; Samson *et al.* 1979) with simple sporophores, campanulate apiculate primary spores containing a relatively small number of nuclei, and a characteristic mechanism of spore discharge among two genera. The application of the mechanism of spore discharge as a generic criterion confirms the narrow definition of *Entomophthora* proposed by Remaudière and Keller (1980) despite the de-emphasis here of the value of spore morphology. The exceptionally high degree of morphological and biological homogeneity of this group of species would be manifestly lowered if the mode of spore discharge did not exclude both *Empusa caroliniana* Thaxter and the species found by Balazy (1978) and discussed above. Both of these species have simple sporophores and plurinucleate primary

spores but are discharged by papillar eversion rather than by the "fungal cannon" mechanism of *E. muscae*.

*Massospora* Peck. This venerable genus which affects gregarious cicadas (Homoptera: Cicadidae) was not treated in the Batko classification until his phylogenetic treatment (Batko 1974) since *Massospora* represents the only genus of the entomogenous Entomophthorales whose validity and circumscription (see Soper 1974, 1981) has never been questioned. The species of this genus have plurinucleate primary spores which are not forcibly discharged from the simple sporophores lining small cavities in the abdomen of affected insects.

*Triplosporium* (Thaxter) Batko, nom. gen. conserv. prop. Batko (1964b) accepted Thaxter's (1888) belief that *E. fresenii* and similar species should be accorded generic status. This small and natural group of species is distinguished primarily by its unique mode of zygosporogenesis and ovoid zygospores with black (or very dark) epispores. Batko (1964b) circumscribed the genus to include species whose primary spores are quadrinucleate, borne on simple sporophores, and capable of producing capillispores as one type of secondary spore. Remaudière and Keller (1980) accept Batko's circumscription of this genus but they (1) reject the requirement for the primary spores to be quadrinucleate in order to justify the (contentious) inclusion of *E. turbinata* Kenneth (1977), and (2) replace *Triplosporium* (Thaxter) Batko with the older generic name *Neozygites* Witlaczil (1885). Humber *et al.* (1981) offer several reasons why *Triplosporium* should be conserved against the older and nomenclaturally correct name *Neozygites*; this nomenclatural issue will be resolved at the 13th International Botanical Congress during the summer of 1981.

*Entomophaga* Batko. This genus, based on *Entomophthora grylli* Fres., was proposed to include species with multinucleate spores and simple sporophores but without forming rhizoids. Its validity has been questioned because of its linkage to the unacceptable *Culicicola* through Batko's emphasis on rhizoids and also because no satisfactory criterion has been proposed to distinguish unambiguously between any of these species and the morphologically similar *Conidiobolus*. The lack of this criterion seemingly prompted Remaudière and Keller (1980) to emphasize spore morphology by transferring all species of *Entomophaga* and those of *Culicicola* was round rather than campanulate primary spores to *Conidiobolus*. However, the criterion of nuclear morphology noted above and discussed fully by Humber (1981b) readily delimits *Conidiobolus* from *Entomophthora* sensu stricto and its segregate genera, and confirms the validity of *Entomophaga* for species with multinucleate unitunicate primary spores containing large, readily stainable nuclei, and discharged by papillar eversion from simple sporophores.

*Zoophthora* Batko and *Erynia* Nowakowski. Batko (1964b) described *Zoophthora*, with *Entomophthora radicans* Brefeld (= *Entomophthora sphaerosperma* Fresenius) as its type, for all species having uninucleate, bitunicate spores borne on branched sporophores and producing rhizoids. This genus has been accepted as a natural and valid grouping. Four subgenera were proposed by Batko (1966) based on the morphology of sporophores, rhizoids, and cystidia, and the type of secondary spores produced. Remaudière and Hennebert (180) limited *Zoophthora* to species capable of forming anadhesive secondary spores atop capillary sporophores (see King and Humber 1981), with all other *Zoophthora* species being transferred to *Erynia* Nowakowski (1881). The Remaudière classification modified the sense of Batko's grouping to accommodate species with simple sporophores and even multinucleate unitunicate primary spores so long as the spore shape was similar to that in other *Erynia* species.

For purposes of discussing the Remaudière classification in this paper, it has been necessary to accept this generic separation on at least a provisional basis. Considerable objection can be raised, as noted above, about the use of capillary sporophores as a character of generic significance. This issue has been treated in part by Ben-Ze'ev and Kenneth (1981a) and will be discussed more fully by these authors in a subsequent paper.

A nomenclatural problem reminiscent of the simultaneous use of *Empusa* Cohn and *Entomophthora* Fresenius as taxonomically distinct genera has been raised by Remaudière and Hennebert's use of both *Zoophthora* Batko and *Erynia* Nowakowski. In 1881, Nowakowski proposed the genus *Erynia* to include *Entomophthora ovispora* Nowakowski (1877) and *Entomophthora curvispora* Nowakowski (1877), but later rejected this generic name in favor of Brefeld's (1877) usage of *Entomophthora* as taxonomically distinct from *Empusa* (Nowakowski 1882, 1883). At the time of its description, *Zoophthora* Batko (1964b) included only its type species, *Entomophthora radicans* Brefeld. However, the inclusion in *Zoophthora* of *E. ovispora* and *E. curvispora* (Batko 1964d) automatically required the adoption of the earlier name. Batko (1966) was incorrect in believing that Nowakowski's later disuse of *Erynia* removed that name from consideration in matters of nomenclatural priority.

The ultimate resolution of this nomenclatural problem with *Erynia* and *Zoophthora* depends on the outcome of the debate to establish a concensus opinion of whether the Batko or Remaudière classification, or some modification of one of them, shall be accepted for the species with bitunicate, uninucleate spores on branched sporophores: If, on the one hand, two genera are recognized with one based on *Entomophthora ovispora*, the type species chosen for *Erynia* by Remaudière and Hennebert

(1980), and the other on *Entomophthora radicans*, there is no nomenclatural problem to resolve. If, on the other hand, common practice rejects and generic separation and recognized only a single genus which includes both *E. ovispora* and *E. radicans*, then either *Erynia* would have to be adopted as the correct generic name or *Zoophthora* might be proposed for conservation against *Erynia*.

It is unfortunate that such a confusing nomenclatural issue should arise one more in the Entomophthorales, but the three possible resolutions are unambiguous. My personal opinion should be clear from this paper: I do not believe that the species originally classified by Batko in *Zoophthora* should be split between two genera separated by characters such as spore morphology and the formation of capillary sporophores. As much as it might prevent some amount of further confusion in the literature if *Zoophthora* Batko were conserved against *Erynia* Nowakowski, I do not believe that such a proposal would be accepted for incorporation in the lists of *nomina conservanda* in the International Code of Botanical Nomenclature. The great preponderance of species proposed as or transferred to *Zoophthora* have already been transferred to *Erynia* by Remaudière and Hennebert (1980) or here; the relative number of species required to be transferred as a consequence of conservation of a younger name against an older and nomenclaturally correct one appears to be one of the major concerns of the nomenclatural committees which decide these matters. The new combinations in *Erynia* proposed here are fully justified and nomenclaturally correct whether one accepts the taxonomy of Batko (and his followers) or of Remaudière and Hennebert for classifying the species in question.

*Strongwellsea* Batko & Weiser emend. Humber. Humber (1976) provided an emended generic description and validation of this genus whose spores are uninucleate and bitunicate but are borne on simple rather than branched sporophores, and are discharged by papillar eversion. Remaudière and Keller (1980) provided no effective rationale for rejecting the importance of sporophore morphology or other diverse supporting data (Humber 1976) in order to synonymize *Strongwellsea* with *Erynia*. There can be little doubt from the criteria considered here to be significant at the generic level that *Strongwellsea* must be recognized as a separate genus. A more extensive support for this opinion is presented by Humber (1981a).

*Culicicola* Nieuwland. This genus is nomenclaturally superfluous since its type species, *Entomophthora culicis* (Braun) Fres., belongs in *Entomophthora* sensu stricto (Remaudière and Keller 1980; King and Humber 1981). The species included in this genus by Batko (1964c-d) are now dispersed to *Entomophthora* s.str. or *Conidiobolus* Brefeld by Remaudière and

Keller, although some question remains whether the conidioboloid species producing rhizoids – *E. apiculata*, *E. major*, and *E. papillata* (which was not classified by Batko 1964d, but clearly belonged in this group) – should be in *Conidiobolus* or in a closely allied but different genus. Batko's inclusion in *Culicicola* of *E. virulenta* (= *C. thromboides*; see Latgé *et al.* 1980) was based on the erroneous description of this species as producing rhizoids (Humber *et al.* 1977).

Batko regarded this as the most tentative of his proposed segregate genera, and he later apparently rejected *Culicicola* in his extended justification of this taxonomic ideas and discussion of phylogeny in the Entomophthorales (Batko 1974). *Culicicola* has been the most objectionable of his genera for other students of these fungi.

*Conidiobolus* Brefeld. Even though Batko and Weiser (1965) note that resting spores of *Conidiobolus* are not budded off to the side as in all other genera considered in the Batko classification, they again cited the widely used but untenable "criterion" which supposes *Conidiobolus* species to be basically saprobes while those of the other genera treated were entomopathogenic. Remaudière and Keller (1980) regard the lack of a suitable criterion to delimit *Conidiobolus* from morphologically similar species put in *Culicicola* and *Entomophaga* by Batko (1965b-d), with round to pyriform, multinucleate primary spores and simple sporophores, to justify their inclusion in a broadly redefined *Conidiobolus*.

As indicated here, however, nuclear cytology does provide the criterion sought to delimit *Conidiobolus* species from those of *Entomophthora* and its segregates (Humber 1981b). Some of the species transferred to *Conidiobolus* from *Entomophthora* sensu lato truly are species of *Conidiobolus*; these include *E. virulenta* (= *C. thromboides*; Latgé *et al.* 1980), *C. obscurus*, and possible *C. apiculatus*, *C. major*, and *C. papillatus* (although these last three species may belong in a separate genus allied to *Conidiobolus* due to marked differences in the structure of the sporophore with the presence of rhizoids as a supporting character. Other species – e.g., *E. grylli*, *E. batkoi*, and *E. gigantea* Keller – have nuclei whose morphology indicates their close affinities to *Entomophthora* than to *Conidiobolus* (Humber 1981b).

*Tabanomyces* Couch, Andreeva, Laird, & Nolan. This little known genus (Couch *et al.* 1979) was described for the peculiar mode of germination and spore formation for entomophthoralean resting spores found in larvae of tabanid flies in the Soviet Union. The thick-walled, colorless, ovoid resting spores of the type species, *T. milkoi* (Dudka & Koval) Couch & *al.*, were originally described as *Coelomomyces milkoi* Dudka & Koval. During the germination of the spores, the single nucleus undergoes two (meiotic?) divisions, and a short, thick, quadrinucleate

sporophore forms. This hypha becomes septate, and each uninucleate cell produces and discharges a lateral primary spore which is, in turn, capable of producing a secondary spore on a short capillary sporophore (Couch *et al.* 1979). These events and structures are essentially identical to those described for the germination of resting spores in the nematophagous genus *Meristacrum* Drechsler (Davidson and Barron 1973). The nuclei illustrated by Couch *et al.* (1979) are relatively small and have a prominent central nucleolus; this type of nucleus resembles that of *Conidiobolus* (Humber 1981b) and *Meristacrum* (Humber, unpublished).

Remaudière and Keller (1980) do not mention *Tabanomyces*, but this genus cannot be easily placed in any entomogenous genus included in the Batko or Remaudière classifications. The remarkable similarity of *Tabanomyces* and *Meristacrum* indicates that these genera are synonymous and that *T. milkoii* must be recognized as a heretofore unknown entomopathogenic species of *Meristacrum*.\*

*Tarichium* Cohn. This genus was created for those species of the Entomophthorales known only by their resting spores (see MacLeod and Müller-Kögler 1970). Neither the Batko nor Remaudière classifications fully accept this genus, but properly regard its members as having still undetermined affinities. It can be assumed that *Tarichium* species may be connected to a species producing primary spores and, thus, placed in the appropriate modern genera. A taxonomic system which accepts a narrow sense of *Entomophthora* and a number of other entomopathogenic segregate genera must also accept *Tarichium* as the correct provisional name for the species involved rather than the name *Entomophthora* (*Tarichium*) as was suggested (without any formal nomenclatural status as a subgenus of *Entomophthora*) by MacLeod and Müller-Kögler (1970).

#### Concluding Remarks on Generic Classification

Despite its manifest shortcomings, the classification proposed by Batko (1964a-e, 1966; Batko and Weiser 1965) forms the basis for all other contemporary approaches to a generic classification for the Entomophthorales. This classification is discussed in depth (although not substantially amplified) in the first attempt to outline the course of evolution in the Entomophthorales (Batko 1974). The Remaudière classification attempted to eliminate the flaws which Remaudière and his colleagues perceived to prevent the general acceptance of the Batko classification. A

generic arrangement more similar to that proposed by Batko than by Remaudière, Hennebert, and Keller emerges here in view of the acceptance of the primary spore karyology, sporophore morphology, and the mode of primary spore discharge as the three characters best suited for delimiting genera. Ben-Ze'ev and Kenneth (1981a, 1981b, and other papers in preparation) also accept the karyology of primary spores and morphology of sporophores as characters with generic importance, but do not recognize the mechanism of spore discharge to be taxonomically significant.

No formal proposal of yet another new generic classification seems appropriate at this time. This study has sought only to evaluate the characters and criteria which have been or might be used to construct generic classifications of the Entomophthorales. I have extended the conclusions of this study regarding appropriate generic criteria to the existing classification schemes in order to show their net effects and to stimulate further discussion and debate.

Each attempt to propose a new generic classification for the Entomophthorales has suffered from the effects of one or more faultily circumscribed genera and from the less obvious effects of incompleteness. It is clear from the still incomplete catalogue of species described as or attributable to *Entomophthora* (*sensu lato*) (MacLeod 1963; MacLeod and Müller-Kögler 1970, 1973; MacLeod *et al.* 1976), that the available information about a distressing number of species is inadequate to allow a reliable classification in the narrowly defined genera comprising the contemporary classification schemes. It must also be noted that all of these latter-day efforts to reclassify the Entomophthorales have concentrated almost exclusively upon the entomopathogenic genera. Several morphologically diverse genera are known only as saprobes or pathogens of fern gametophytes, desmid algae, tardigrades, and nematodes; at least three species of this order are also known to affect humans and other vertebrates (King 1979). These fungi are no less valid members of the Entomophthorales than those species attacking insects; it is inappropriate to argue about the choice of criteria used to define genera or about the correctness of one or another classification of the Entomophthorales without taking these non-entomogenous genera into full account.

Despite these impediments to the proposition of a comprehensive generic scheme, however, I believe that most concerned parties now agree that following a period of further discussion of taxonomic criteria, it will be possible to propose a generally acceptable, realistic, and phylogenetically based classification for the Entomophthorales in which each genus will have a uniformly high degree of morphological and biological homogeneity. Despite the obvious difficulties in proposing such a comprehensive scheme, some comfort should be found in the thought that IF the

\* *Meristacrum milkoii* (Dudka & Koval) Humber, comb. nov., basionym: *Coelomomyces milkoii* Dudka & Koval in Dudka, Koval & Andreeva, 1973, *Novitates Systematicae Plantarum non Vascularium* 10, 88-91. Synonym: *Tabanomyces milkoii* (Dudka Koval) Couch, Andreeva, Laird & Nolan, 1979, *Proc. Natl. Acad. Sci. (USA)* 76, 2299-2302.

generic scheme which is yet to emerge is accurate, then no further major adjustments of generic circumscriptions should be necessary. As new taxa are found and described, and as older taxa being held in abeyance become better known, they will be classified in

the existing stable generic structure or seen to differ in enough significant characters to warrant the erection of new genera.

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